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TUBERCLE BACILLI IN THE BLOOD.*

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Notwithstanding the large number of investigators who have made a study of the presence of tubercle bacilli in the circulating blood, it is nevertheless a fact that the most striking point about the results so far obtained is the total lack of agreement between the reports of the different workers.

In a number of cases there is an absence of sufficient detail as to technic and controls, but leaving these records out of account there remain a long series of reports in which, after the most careful and painstaking work described in minute detail, the authors have come to absolutely contradictory conclusions.

A difference so marked could not fail to attract attention, and within the past year a number of extensive reviews of the literature have been made by different writers, especially by Bacmeister and Reuben, Elsaesser, and Rothacker and Charon.

As the majority of these articles are in German, and so far as known there is no corresponding record in English, a brief résumé of the principal articles is here given, including some of the most recent work on the subject.

The first investigator in this field seems to have been Villemin, who in 1868 obtained positive inoculation results with the blood in miliary tuberculosis. Weichselbaum, in 1884, found tubercle bacilli microscopically in otherwise sterile blood from miliary tuberculosis. Next followed Meissel, Hildebrand, Huguenin, Sticker, Gary, Bergeron, Lüdke,[1] Lustig, Rutimeyer, and others who, either microscopically or by animal inoculation, demonstrated the presence of tubercle bacilli in the blood in a varying percentage of severe cases.

As a result of this work it became recognized that bacilli could be found in the blood irregularly in the last stages of miliary tuberculosis, with a possibility that the same might be true of certain severe pulmonary infections, tho the generally accepted opinion was expressed by Bergeron in 1904 when he stated that tubercle bacilli are found in the blood in miliary tuberculosis, but only in relatively rare cases, and that in non-miliary tuberculosis, even of a rapid type, bacillemia does not exist.

A greatly added impetus was given to the investigation by the introduction of the Stäubli acetic acid method in 1908, followed by the Schnitter modification in 1909.

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[1] *Wien. klin. Wchnschr.*, 1906, 19, p. 949.

The Stäubli-Schnitter method is as follows: 10–15 c.c. of blood from the vein are added to a like amount of 2–3 per cent citric acid or double the amount of 3 per cent acetic acid, then carefully shaken and allowed to stand for one-half hour. The fluid is centrifuged and pipetted off, and 1 c.c. of water is added to the sediment. Then 2 to 5 times the amount of 15 per cent antiformin is added and the mixture shaken. As soon as solution is complete, which happens very quickly, the mixture is again centrifuged, the fluid pipetted off, and the slight deposit is washed and examined by the Ziehl-Nielson stain.

Schnitter[1] found acidfast bacilli in 31.6 per cent of 38 cases of tuberculosis. He found very few organisms, not more than 5 or 6 in most cases, and the majority of these were "granules." Schnitter came to the conclusion that tubercle bacilli are probably regularly present in the blood in organic tuberculosis, but that this does not necessarily lead to miliary infection, since the latter is due to a sudden explosive outburst of organisms into the blood in such numbers that the natural protective powers of the organism are unable to cope with them.

Just previous to the work of Schnitter, Rosenberger,[2] in this country, had published his report of 100 per cent positive microscopic results in 49 cases of tuberculosis of all stages, 5 miliary. Rosenberger drew blood into sterile sodium citrate, centrifuged and examined the sediment. In a later report Rosenberger brought his list of cases up to 300, in all of which he claimed to have found acidfast bacilli in the blood.

Schroeder and Cotton,[3] Burvill-Holmes,[4] Anderson, and other American workers attempted to repeat Rosenberger's work, using the same method, but utterly failed to reach the same conclusions, and a like result was reached by the English investigators, Hewat and Sutherland,[5] working with cattle, altho Forsyth[6] in England, with this method, reported 100 per cent positive results in 10 open cases of tuberculosis and negative results in 2 closed cases.

The work of Anderson[7] was especially interesting on account of his inoculation and culture tests. All direct microscopic examinations of blood were negative and of 83 guinea-pigs inoculated intraperitoneally with human blood none developed tuberculosis, but the blood of 13 tuberculous guinea-pigs gave one positive result when injected into other guinea-pigs, while of 8 infected rabbits the blood of 7 produced tuberculosis in guinea-pigs. In 3 cases rabbit-blood specimens gave cultures of tuberculosis, this being the first time, according to Anderson, that successful cultures have been reported from rabbits' blood.

With the finding by Brem,[8] and afterward by others, of acidfast bacilli in laboratory distilled water it was thought that a probable explanation could be given of the result reported by Rosenberger. Since then but little has appeared upon the subject in this country, but a large amount of work has been done in foreign laboratories, especially among the Germans.

Lippmann,[9] with the Stäubli-Schnitter method and new slides, found 11, or 44 per cent, positive in 25 pulmonary cases, all stages. He found very few bacilli and no connection between fever and positive blood test.

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[1] *Deutsch. med. Wchnschr.*, 1909, 35, p. 1566.

[2] *Am. Jour. Med. Sc.*, 1909, n.s., 137, p. 267.

[3] *Bull. 116, Bureau of Animal Industry, U.S. Dept. Agric.*, 1909.

[4] *Am. Jour. Med. Sc.*, 1910, 139, p. 99.

[5] *Brit. Med. Jour.*, 1909, 1, p. 1119.

[6] *Ibid.*, p. 1001.

[7] *Bull. 57–59, Hyg. Lab., U.S. Pub. Health Serv.*, 1909, p. 7.

[8] *Jour. Am. Med. Assn.*, 1909, 53, p. 909.

[9] *München. med. Wchnschr.*, 1909, 56, p. 2214.

Jessen and Rabinowitsch,[1] with a slightly modified Stäubli-Schnitter method, and all precautions as to slides, glassware, etc., had 10, or 28 per cent, positive results in 36 cases, different stages. Ziehl, Much, and Gram stains were used, and "granules" were included among positive results. The Ziehl stain gave best results. No agreement was evident between the clinical stage and the presence of bacilli.

Acs-Nagy,[2] with the Stäubli-Schnitter method, and careful aseptic precautions, found 11, or 45 per cent, positive blood specimens in 24 cases, of which 5 were miliary, the remainder pulmonary tuberculosis of the second and third stages. The majority of organisms were "granules." No agreement found between microscopic result and severity of case, or time before death.

Kurashige,[3] with a modified Stäubli-Schnitter method and Ziehl stain, reported positive blood findings in the astonishing ratio of 100 per cent in 155 cases, all stages. No details given as to asepsis and controls. Bacilli were present in great numbers, as many as 30 in one field. Besides the above cases of pulmonary tuberculosis, Kurashige examined the blood of 34 apparently healthy subjects and found acidfast bacilli in the blood of 20, or 59 per cent. In 3 of these cases, tuberculosis developed during the next 8 months. Guinea-pigs inoculated with microscopically positive blood from 4 apparently normal persons developed tuberculosis. In a later paper Kurashige[4] gave the results of a series of repeated examinations of blood at intervals of 1 to 2 weeks. Of the 20 cases, all stages, from whom the blood was taken, each gave positive results, and of the 114 specimens of blood, 104 were positive. None were tested in animals. Kurashige concludes that tuberculosis is primarily a bacillemia, with later localization in the tissues and the appearance of characteristic symptoms.

Sturm,[5] seeking to confirm Kurashige's findings, came to quite different conclusions. With corresponding preparations, the one stained by Much, the other by the Ziehl-Nielson method, he found by the former stain 42 per cent and by the latter only 22 per cent positive in 50 cases, all stages. Of 10 specimens from apparently normal persons, all were negative. Animal tests, however, made by the inoculation of 5-6 c.c. of blood intraperitoneally in guinea-pigs, gave the large number of 46 per cent positive results. There was no agreement between the presence of bacilli in sputum and in the blood, or between the positive blood test and the severity of the case.

Krause-Hannover,[6] using the Uhlenhuth antiformin method, had 11 per cent positive results in 32 severe cases of the second and third stages. A second series of 100 cases, all stages, gave 22 per cent positive results. Many cases positive at one time were found negative at another, and the presence of bacilli was no reliable guide for prognosis. Animal tests were not made.

Suzuki and Takaki[7] examined a great series of blood specimens and compared the results with the Pirquet test. They found acidfast bacilli by the Stäubli-Schnitter method in 98.5 per cent of 517 tuberculosis cases of all stages, and also, in 51.85 per cent of 54 apparently healthy subjects. Reagents and all materials were sterilized. Both Ziehl and Much stains were used, but the latter method was discarded as unsafe. Animal tests were apparently made, but there is only a passing allusion to positive results, no details being given.

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[1] *Deutsch. med. Wchnschr.*, 1910, 36, p. 1116.

[2] *Wien. klin. Wchnschr.*, 1910, 23, p. 1313.

[3] *Ztschr. f. Tuberk.*, 1911, 17, p. 347.

[4] *Ibid.*, 1912, 18, p. 430.

[5] *Beitr. z. Klin. d. Tuberk.*, 1911, 21, p. 239.

[6] *Ztschr. f. Tuberk.*, 1911, 17, p. 436.

[7] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1911, 61, p. 149.

The writers were impressed by the agreement between the microscopic test and the skin reaction. With all persons who gave a positive skin reaction there was also a positive blood test.

Liebermeister,[1] in over 100 tuberculous cases of different stages, obtained positive inoculation results in 48 per cent. By the Stäubli-Schnitter microscopic method, with Ziehl stain and aseptic precautions, he found acidfast bacilli in each of 15 cases of open tuberculosis, and in 11 of 13 cases of closed tuberculosis. In more than 70 cases, not clinically positive, several cases of scrofula, and also in a long series of patients in various diseased conditions, not tuberculous, acidfast bacilli were found. He concludes that these conditions have some connection with tuberculosis not understood, and suggests a "secondary" tuberculosis, analogous to the secondary stage in syphilis. Liebermeister notes that when several guinea-pigs are inoculated at the same time with the same dose only one or two become tubercular. Therefore one or two negative inoculation results do not exclude tuberculosis.

Rumpf,[2] together with Zeissler, used the Schnitter method with oxalate in place of citrate, and a concentrated antiformin. All aseptic precautions were observed. He found acidfast bacilli in 100 per cent of 25 cases of tuberculosis, also in 6 former patients healed for 20 years, and in 7 healthy persons, one of whom was scrofulous as a child. Of 35 guinea-pigs inoculated intraperitoneally with microscopically positive blood, only 3 became tubercular. In 2 of these the blood came from light-closed cases, in the third from an open third-stage case.

Hilgermann and Lossen,[3] using the Stäubli-Schnitter method and all precautions with glassware and solutions, found acidfast bacilli in 17, or 25 per cent, of 64 lung cases, all stages, but made no animal tests. Authors found no connection between positive blood and temperature conditions. The course of the disease was less favorable in the majority of the cases with positive blood, but this could not be attributed to a beginning miliary process since in many cases the positive blood test was followed by a temporary improvement.

Kennerknecht[4] examined blood in 120 children, tuberculous, tuberculosis suspects, and apparently healthy. Using the Stäubli-Schnitter technic with the addition of absolute alcohol, the Ziehl and Gram stains, new slides, and all precautions with glassware and reagents, acidfast bacilli were found in 109, or 91 per cent, of all specimens. Very few bacilli, sometimes more in the beginning or healing stage than at the height of disease, and "granules" included in positive findings. Of 13 guinea-pigs inoculated intraperitoneally all are said to have become tuberculous, but doubt is thrown upon the autopsy results by Bacmeister and also by Rabinowitsch.

Klemperer,[5] with the Stäubli-Schnitter method and Ziehl stain, examined blood in 39 cases of tuberculosis, suspected tuberculosis, other diseases, and healthy persons. He found acidfast bacilli in the majority of tuberculosis cases and tuberculosis suspects and in one case of liver cirrhosis, but never in healthy persons. Only characteristic well-stained bacilli counted. These were generally few in number. Full details of aseptic precautions with solutions and glassware. No animal tests. Klemperer, observing that blood free from bacilli dissolves in antiformin more slowly than blood in which bacilli are found, argues from this a probable lessened resistance. In opposition to Suzuki and Takaki positive blood results were not found in all cases giving positive Pirquet reaction. Klemperer thinks the finding of bacilli of worth for diagnosis, but not for prognosis.

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[1] *Med. Klin.*, 1912, 8, p. 1018.

[2] *München. med. Wchnschr.*, 1912, 59, p. 1951.

[3] *Deutsch. med. Wchnschr.*, 1912, 38, p. 895.

[4] *Beitr. z. Klin. d. Tuberk.*, 1912, 23, p. 265.

[5] *Therap. d. Gegenw.*, 1912, 53, p. 433.

Ranström-Upsala[1] used the method of Rosenberger and the Uhlenluth antiformin method, sterile glassware, and all aseptic precautions. Of 36 cases of lung tuberculosis, 9, or 25 per cent, showed acidfast bacilli. This author alone seems to have found a definite ratio between temperature and positive blood test.

Bacmeister and Reuben,[2] with the Stäubli-Schnitter method, sterile glassware, and filtered fluids, examined blood from "a great series" of cases of lung tuberculosis in the third stage, from 15 cases in the first stage, from 8 non-tuberculous subjects, and from a number of sound rabbits. In all of these specimens acidfast bacilli were found. Granules were not counted. Blood from 15 light and severe cases was inoculated into rabbits and in part into guinea-pigs. These tests gave only negative results. The authors came to the conclusion that the chemical reaction of acetic acid and antiformin in the Stäubli-Schnitter method may give rise to appearances which simulate tubercle bacilli.

Lydia Rabinowitsch[3] criticized the use of rabbits by Bacmeister instead of guinea-pigs which she found much more suitable for the study of human tubercle bacilli. She also questioned the finding of bacilli in normal rabbits by Bacmeister and by Sturm. Rabinowitsch made the interesting observation that the blood of highly tuberculous animals frequently fails to produce tuberculosis when injected into guinea-pigs, but that after the tuberculous animals have been injected with tuberculin the blood then gives a positive inoculation result. She quotes the experience of Suzuki and Takaki in this connection, and also the observations of Virchow and Orth published 22 years before, and agrees with the latter authors that the above result is probably due to the "mobilizing" of the bacilli through the action of the tuberculin.

On the above point Bacmeister[4] in a new series of tests comes to the same conclusion as Rabinowitsch. In 15 cases of light tuberculosis he found acidfast bacilli, but with all of these specimens animal tests were negative. Upon injecting the patients with tuberculin and taking blood at the height of the reaction he was able to produce typical tuberculosis in 4, or 13.3 per cent, of 30 inoculated animals. The acidfast bacilli found in his earlier work with rabbits and guinea-pigs he later considered to have been "Futterungs" bacilli.

Rosenberg[5] examined blood in a series of surgical as well as pulmonary cases, also in healthy persons. In the healthy he found nothing in any case, but in his other tests acidfast bacilli were found in nearly every specimen. Very few organisms were present in any case and these found after long search. There was no definite relation between severity of disease and bacilli in blood, and the latter condition is considered of no value for prognosis, tho thought to be specific as a means of diagnosis.

Nobécourt and Darré[6] studied the question of bacillemia in tuberculous children of various ages with different forms of tuberculosis, using exclusively the method of guinea-pig inoculation. Ten to 20 c.c. of blood were divided into equal parts and inoculated intraperitoneally into 2 or 3 guinea-pigs.

[1] *Deutsch. med. Wchnschr.*, 1912, 38, p. 1535.

[2] *Ibid.*, p. 2350.

[3] *Berl. klin. Wchnschr.*, 1913, 50, p. 110.

[4] *München. med. Wchnschr.*, 1913, 60, p. 343.

[5] *Ibid.*, p. 404.

[6] *Bull. Soc. d'étud. scient. sur la Tuberc.*, 1912, 2, p. 176.

Of 40 patients, in acute, subacute, and chronic stages, only 4 gave positive results. The first, a child of 4 years, showed a clinical picture of general tuberculosis. A guinea-pig, inoculated intraperitoneally, died 12 days later and showed fresh granulations in the spleen containing acidfast bacilli. A second animal inoculated with a fragment of this spleen developed general tuberculosis. Another specimen of blood taken 8 days later, while the fever was still high, gave only negative results in 2 guinea-pigs, 1 inoculated subcutaneously, the other intraperitoneally. This patient was later discharged, apparently completely cured, and the diagnosis would have remained in doubt but for the positive animal test.

The second positive case was a little girl of 4 years with general tuberculosis of an extremely rapid type, the pulmonary symptoms predominating. Two guinea-pigs inoculated intraperitoneally developed tuberculosis.

The third positive blood case was in a boy of 9, with typical tuberculous meningitis. Blood taken 3 days after beginning of disease was negative in 2 pigs, but a second specimen taken 6 days later produced general tuberculosis by intraperitoneal inoculation.

The fourth positive blood was from an infant with acute tuberculous broncho-pneumonia. Two inoculated guinea-pigs developed general tuberculosis.

The authors point out the opposite inoculation results with different blood specimens from the same patient, and they emphasize the importance of the period of the disease when the specimen is taken, considering this a more essential point than the quantity of blood inoculated.

Querner[1] used only animal tests. He injected guinea-pigs intraperitoneally with sediment obtained by the Stäubli-Schnitter method from the blood of 37 chronic pulmonary cases in all stages. In 3 cases two inoculations were made, making 40 tests in all. The animals, killed 27–143 days after inoculation, proved to be negative in every instance. The authors made control tests of the action of antiformin on tubercle bacilli, and found no lessening of virulence produced.

Krabbel[2] made microscopic examinations of blood in 35 cases of tuberculosis of lung, third stage, tuberculosis of bones, of glands, or of skin. Using Stäubli-Schnitter method, new slides, sterile glassware, and stains constantly renewed, he found acidfast bacilli in 20, or 57.1 per cent. No animal tests made. Krabbel finds no significance in positive blood tests as they may be negative in advanced cases, and positive in cases getting well.

Brandes and Mau[3] confined their investigations to surgical cases. With the Stäubli-Schnitter method and Ziehl stains, they examined blood in 40 cases of surgical tuberculosis of all stages (height and beginning of disease, and clinically healed). They used sterile glassware, new slides, and water freshly distilled, and found acidfast bacilli in 18, or 45 per cent. Bacilli in blood were thought to indicate tuberculosis, but the test was not considered of practical value.

De Verbizier[4] examined blood in 15 cases of tuberculosis by the Stäubli-Schnitter and Rosenberger methods, but found no acidfast bacilli in any case. Fifteen guinea-pigs inoculated intraperitoneally were all negative. The author concludes that bacillemia must be very rare in tuberculosis.

Rogers and Murphy,[5] using the Stäubli-Schnitter and absolute alcohol method, tested blood in 50 cases of pulmonary tuberculosis, all stages, and in 5 apparently normal persons. They found acidfast bacilli in all, morphologically identical with tubercle bacilli. Authors made numerous control tests. The water used was tested by adding albumen, centrifuging one-half to one hour, and examining the sediment, with results uniformly negative. A small loopful of tuberculous culture was then added to 1 c.c. of water and a little albumen. After the mixture was carried through the same process as test specimens, the tubercle bacilli appeared on the slides in clumps. To eliminate crystals the authors made a series of tests with combinations of acetic acid, alcohol and albumen, acetic acid and solutions of alkalies and antiformin, but none of these tests gave crystals at all like the appearances found in the blood. These results are of interest as they are in opposition to the suggestion of Bacmeister and Reuben.

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[1] *München. med. Wchnschr.*, 1913, 60, p. 401.

[2] *Deutsch. Ztschr. f. Chir.*, 1913, 120, p. 370.

[3] *Deutsch. med. Wchnschr.*, 1913, 39, p. 1137.

[4] *Rev. de méd.*, 1913, 33, p. 161.

[5] *Jour. Am. Med. Assn.*, 1913, 60, p. 995.

Dreesen[1] tested blood in 70 cases of tuberculosis, tuberculosis suspects, and apparently normal persons. Blood was drawn into freshly distilled water, kept in the dark until next day, and then centrifuged for 1 hour. One-half of the sediment was treated with antiformin for 2–3 minutes, diluted with freshly distilled water, centrifuged, and sediment examined with Ziehl stain. Only well-stained rods counted. Acidfast rods and granules were found in 74 per cent of the surely tubercular, 55 per cent of the suspicious cases, and 46 per cent of the apparently normal cases. In marked contrast with these findings was the result of animal tests. The other half of the blood sediment described above was emulsified with 2 c.c. of sodium chlorid and injected into 2 guinea-pigs, one pig being inoculated in the groin, the other in the peritoneum or in the liver. Of 128 pigs thus treated 39 died prematurely, the other 89 lived 7–8 weeks, and only one of all these animals gave any evidence of tuberculosis. This pig, inoculated from a case of advanced phthisis, showed true tuberculous lesions, and tubercle bacilli in smears. Tuberculin tests of all the other animals were negative.

Fränkel[2] made examinations in 25 cases including tuberculosis second and third stages, catarrh, and other conditions. Eighteen specimens of blood were examined microscopically by the Stäubli-Schnitter method, Ziehl, Much, and Spengler stains. Four of these specimens showed acidfast bacilli. Forty-two guinea-pigs were inoculated subcutaneously and intraperitoneally and of these only 2 developed tuberculosis. The blood injected in 1 pig was from a case of advanced phthisis, but in the other animal the blood was from a case of chronic bronchitis with no evidence of tuberculosis, and with blood and sputum negative microscopically.

Bernard, Debré, and Baron,[3] after trying various methods, selected the following for their work. Ten cubic centimeters of blood were taken from the vein into a sterile tube containing 20 c.c. of 30 per cent alcohol. Complete laking was obtained by the gradual addition of about 30 c.c. of 40 per cent alcohol, with vigorous shaking. After centrifuging half an hour, the fluid was pipetted off. The sediment was redissolved in 40 c.c. of 40 per cent alcohol, shaken energetically, and 1–2 drops of 10 per cent alcoholic solution of soda added. The fluid thus obtained is clear and but slightly viscous, if an excess of soda is avoided. The deposit obtained by centrifuging is then examined by the Ziehl stain.

The authors claim many advantages for this as compared with other methods. They examined 41 specimens of blood from 36 tuberculous patients in all stages.

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[1] *Med. Klin.*, 1913, 9, p. 580.

[2] *Deutsch. med. Wchnschr.*, 1913, 39, p. 737.

[3] *Bull. Soc. d'étud. scient. sur la Tuberc.*, 1912, 2, p. 154.

Thirty-seven specimens were negative. In 4 cases the results were completely or partially positive. In the first case the blood was taken from a patient with tuberculous meningitis 3 days before death. Three apparently typical bacilli were found. Of 2 guinea-pigs inoculated, 1 was negative 3 months later; the second developed tuberculous lesions containing acidfast bacilli. Another pig inoculated with gland material from this animal died prematurely. The second positive blood specimen was from a case of miliary tuberculosis and showed a mass of apparently typical bacilli. Guinea-pigs inoculated died accidentally. The third specimen was from a case of pulmonary tuberculosis with cavities. Acidfast bacilli were found on the accidental death of an inoculated animal. In the fourth case the blood was from a patient with miliary tuberculosis, and the inoculated guinea-pig developed typical lesions.

The authors question the accuracy of the methods usually employed, and especially the use of antiformin, as they have found acidfast granules in the deposit at the bottom of a bottle of antiformin, and they suggest the possibility that acidfast organisms may vegetate in this fluid.

Bernard, Debré, and Baron[1] made a second series of experiments with animals experimentally infected. They selected the guinea-pig because it was possible to inject the entire blood of an animal into another. An inoculation dose was prepared by grinding up one centigram of a glycerin potato culture of tubercle bacilli in a sterile mortar, adding 10 c.c. of sterile salt solution, and shaking into a homogeneous emulsion. Of this emulsion 1 c.c., equaling 1 milligram of culture, was then injected into the external jugular vein of guinea-pigs. Great care was used to avoid infecting the surrounding tissues, and the most careful technic was employed in the second part of the experiment when the blood was recovered from the carotid artery and introduced by transfusion into other guinea-pigs. Of 23 guinea-pigs injected with the emulsion all sickened very quickly and severely, and showed the lesions of general miliary infection. The blood of these pigs, drawn at intervals of from 20 minutes to 29 days, produced tuberculosis in every instance when introduced into other guinea-pigs, and the microscopic blood examination was also positive in every case. Inoculations were also made of the urine and bile which had been collected with every aseptic precaution from the infected pigs. Leaving out the animals which died prematurely, all the urine inoculations were positive (pigs inoculated with urines obtained 2, 8, 10, 16, 21, 22, and 29 days after injection), and 5 out of 8 inoculations of bile were positive (bile obtained 2, 16, 20, 21, and 29 days after injection).

In 15 guinea-pigs injected with the same dose of tubercle bacilli subcutaneously the blood was found to be much less virulent, 5 out of 16 guinea-pigs being positive with blood drawn at the seventh hour, 12, 18, 22, and 31 days later. Authors conclude that after intravenous inoculation with tubercle bacilli the organisms remain constantly present in the blood until the death of the animal. The same is true of the urine, probably, but not proven with regard to the bile. Bacillemia inconstant after subcutaneous injection.

Rist, Armand-Delille, and Lévy-Bruhl[2] question the results obtained by Bernard, Debré, and Baron. Authors made tests in 50 cases using the Stäubli-Schnitter method with Kurashiga modification, 1 c.c. of blood examined microscopically by Ziehl and Much stains, and 9 c.c. injected intraperitoneally into guinea-pigs. Of 50 cases, 40 gave a negative result by Ziehl and by Much, and 7 were positive sometimes by Ziehl and sometimes by Much. Guinea-pigs inoculated from these 7 cases were all negative. Three specimens were positive both by Ziehl and by Much. In the first case the blood was from a patient with a cavity. Guinea-pig killed in 2 months showed no sign of tuberculosis. In the second case, the guinea-pig showed apparent lesions of peritoneal tuberculosis and granulations in liver and spleen, but a second guinea-pig inoculated with this material remained healthy. In the third case, the guinea-pig showed lesions of tuberculosis, and a few doubtful bacilli were found in smears. Authors point out the contrast between the work of Bernard, Debré, and Baron, and their own results in which they have but one positive case among 50 tested. They are willing to admit a more or less rapid and transitory dissemination of the bacilli in certain cases, but consider this due to a condition of allergic sensibility. This condition, common to all the adult tuberculous, is incompatible with a septicemia. The extraordinary percentage of bacilli in blood reported by some is thought by the authors to be due to technical errors.

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[1] *Bull. Soc. d'étud. scient. sur la Tuberc.*, 1913, 3, p. 52.

[2] *Ibid.*, p. 19.

During the last few months there have been three important contributions to this subject in articles by Elsaesser, Rothacker and Charon, Lange and Lindemann.

Elsaesser,[1] after an extensive review of the literature, gives the results of his own tests. He examined blood in 41 cases of pulmonary tuberculosis, generally of a severe type, the blood being obtained not long before death. Many different methods and stains were employed, but acidfast bacilli were found in only 3 cases, and in these the Zeissler modification of Schnitter's method was used together with Ziehl's stain. In 25 cases guinea-pigs were inoculated with 1 c.c. of blood, 2 animals being used, one injected intraperitoneally, the other subcutaneously. Each pig was examined as it died. If both of a pair remained alive for some months one was killed and examined. In all cases these tests gave negative results.

Elsaesser calls attention to the strongly contrasting results of different workers, and also to the general discrepancy between the microscopic findings and the results of animal tests. He warns against conclusions based upon the microscope alone since the presence of tubercle bacilli in the blood is not proven without successful animal inoculation. At the same time, even with true bacilli, this proof may not always be obtainable, since if the bacilli should be present in very small numbers the organism might be able successfully to cope with them.

Rothacker and Charon[2] give a synopsis of the investigations of many authors as to the question of bacillemia in the tuberculous, and report upon their own work in the examination of 46 blood specimens from tuberculous patients in all stages. They used the Stäubli-Schnitter method and Ziehl stain, with every safeguard as to glassware, instruments, solutions, etc. As an additional precaution the smears were not dried with filter paper, and many controls of all materials were made. In particular, control tests were made, suggested by the work of Kahn with blood corpuscles, Kahn having stated that the stroma of red blood corpuscles, owing to their content of cholesterin and lecithin, were more or less acidfast in nature, and thus might give rise to confusion with tubercle bacilli. The authors confirmed Kahn's statement as to the acidfast property of lecithin and cholesterin, but found this to be weak in quality, and did not agree with Kahn as to the possibility of confusion with tubercle bacilli. Of the 46 blood specimens examined all specimens from patients in the first stage were negative; about 13 per cent of those from the second stage, and 60 per cent of those from the third stage were found to contain acidfast bacilli. Forty-six guinea-pigs were inoculated, but only one developed tuberculosis, this pig having been injected with blood from miliary tuberculosis. The authors conclude that the results of the Stäubli-Schnitter method are unreliable, and that animal inoculation is the only safe test for the blood.

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[1] *Beitr. z. Klin. d. Tuberk.*, 1913, 26, p. 367.

[2] *Centralbl. f. Bakteriol.*, I. *Orig.*, 1913, 69, p. 478.

Lange and Lindemann,[1] in a paper read before the recent meeting of the Freien Vereinigung für Microbiologie in Berlin, gave the results of their tests upon blood specimens from 78 cases of pulmonary tuberculosis in all stages, 2 tuberculosis suspects, and 2 healthy persons. The authors were able to report only on the microscopic work as the animal tests, begun some months before, were still in progress. Zeissler's modification of Schnitter's method was used, together with Ziehl's stain, and full details are given of the most extreme care with glassware, solutions, instruments, etc. A thorough search was made for one to one and one-half hours on each slide, in over one-third of the specimens both investigators working together, and controlling each other, but all examinations were absolutely negative. Not only were there no acidfast bacilli found, but no definite bacilli of any sort.

In contrast to Bacmeister's statement the blood of 4 normal rabbits, examined in the same manner, also gave entirely negative results.

As a control the authors made suspensions of tubercle bacilli in varying degrees of dilution in guinea-pig blood, then centrifuged and found that in the dilution of 1 to 100 millions the bacilli were still to be found microscopically. In the dilution of 1 to 500 millions they were no longer detected. This "negative" sediment was injected into guinea-pigs, still living at the time of report.

Of the 250 guinea-pigs inoculated in the original blood tests 38 had died, but only one of these had given any evidence of tuberculosis, and in this animal a careful search failed to show tubercle bacilli or any acidfast appearances. The authors conclude that the many reports of positive microscopic results are to be received with much skepticism.

An interesting discussion with opinions both in support and in opposition followed the above paper.

EXPERIMENTS.

Through the courtesy of Dr. Wilson, and the physicians stationed at North Brother's Island, specimens of blood were obtained with Miss Smeaton's assistance from patients in the second and third stages of tuberculosis, all giving a history of tubercle bacilli in the sputum.

A recent study of tuberculous sputa with Miss Smeaton by the Kinyoun ligroin and sodium hypochlorite method as compared with the Elliman-Erlandsen method had shown the former to be an excellent method for the detection of tubercle bacilli in sputum, and as a series of control tests had proved it to be of equal value for the finding of tubercle bacilli in blood specimens, this was the method chosen for the present work.

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[1] *Centralbl. f. Bakteriol., Ref.*, 1913, 57, Beiheft, p. 285.

The technic pursued was as follows: with aseptic precautions from 3–5 c.c. of blood were drawn from the vein at the elbow into a sterile tube containing 2 c.c. of 10 per cent sodium citrate solution. To the blood and sodium citrate in the test tubes was added about three times the amount of sodium hypochlorite of such dilution as to contain 0.56 per cent of available chlorin. One cubic centimeter of ligroin was next added, the mixture thoroughly shaken and allowed to stand over night at room temperature, when examination was made of the lower surface of the sharply separated layer of ligroin. The centrifuge was not used, as originally recommended by Dr. Kinyoun, for in our work with sputum we had demonstrated, by numerous control tests, that if tubercle bacilli were present in the specimen they would be found without previous centrifuging in the soap-like stratum on the lower side of the ligroin layer, and in some cases our results had been found to be less favorable with the centrifuged portion of a specimen than with that portion which had simply stood over night after shaking.

New slides, which had been carefully prepared by boiling in soda solution, rinsing in water, rubbing with alcohol, and thoroughly firing in the flame, were covered with as much as possible of the material to be examined, the smears dried in the air, fixed by moderate heat, and stained with the strong solution of carbol fuchsin recommended by Dr. Kinyoun:

4 gms. fuchsin, basic (Grubler)
8 gms. acid carbolic C.P. crystal
20 c.c. alcohol 95 per cent
100 c.c. water

Decolorization was obtained with 3 per cent hydrochloric acid in 95 per cent alcohol. Methylene blue was used as a counter stain.

All glassware was specially cleaned and sterilized and all solutions of every kind coming in contact with the specimens were passed through the Berkefeld filter until it became evident that the latter precaution was not necessary when unfiltered fluids were used as with the customary routine staining for tubercle bacilli.

Control tests were made during the entire course of the work by means of tubercle bacilli from pure cultures suspended in sterile salt solution. When dilute suspensions of these organisms were added in very small quantities to tubes of sterile blood together with ligroin and hypophosphite, and the further process carried out exactly as with the test specimens, the bacilli were invariably found to be sharply localized beneath the ligroin layer, and could be readily demonstrated in smears even after several days in the hypochlorite mixture.

In all, blood specimens were taken from 50 tuberculous patients, two specimens obtained from one individual, one month apart, making a total of 51 specimens examined. Of these, 13 were from patients in the second stage, and 38 from patients in the third stage of the disease, 4 of the latter being bed patients. Six patients have since died, the first 3 at intervals of from 17 days to 1 month, the last 3 in from 1 month to 5 weeks after the taking of the blood specimens.

The most careful search was made for tubercle bacilli, from 150 to over 300 microscopic fields being examined with each specimen, but in all cases the results were absolutely negative, no acid-fast organisms of any kind being found in the smears. A few rods were occasionally seen, but never one which showed acidfast staining, notwithstanding the precaution of having at least one smear from each specimen overstained and only so slightly decolorized that even partially acidfast organisms must have been detected if present.

Owing to the invariably negative microscopic results no animals were inoculated, as it had been thought best to wait for some positive indications from the microscopic work before beginning animal tests.

As a preliminary to this work, examinations of blood taken from normal animals and human beings were made by the same method. In this way specimens from 13 horses, 9 goats, and 3 laboratory workers were examined with negative findings in all cases.

To summarize, the results of the present work are as follows: a total of 51 specimens from the lung were all negative. Of these, 38 specimens were from the third stage and 13 from the second stage. All the specimens from normal controls, including 13 horses, 9 goats, and 3 humans, were negative.

CONCLUSIONS.

The entirely negative results obtained during the present work by the microscopic examination of 51 specimens of blood taken from patients in advanced stages of tuberculosis are in agreement with the experience of a large number of observers. In other cases acidfast bacilli have apparently been found in the blood of tuberculosis patients by different investigators, but such bacilli have been reported present in normal as well as tuberculous individuals. Since organisms morphologically identified with tubercle bacilli are present occasionally in water and elsewhere it is fair to assume that some, at least, of the acidfast bacilli observed in blood were not tubercle bacilli, and where there are no confirmatory animal tests the proportion of specimens containing true tubercle bacilli must remain an open question.

Not only are animal tests indispensable in all cases before positive microscopic results can be accepted, but it is evident that in all microscopic work the utmost care must be exercised as to filtration of solutions and sterilizing of glassware; also care must be taken that no acidfast organisms attach themselves to the slides during the various processes of fixing and staining.

Tho the animal tests included in the above summary of the work of different investigations are characterized by a striking lack of agreement, in some instances according to the testimony of well-known observers, the results indicate that bacillemia is occasionally present in tuberculosis in a wider range of cases than was formerly supposed. In view of the findings of Sturm, Rumpf, Liebermeister and others it would seem that there must be some modification of the generally accepted opinion that tubercle bacilli are to be found in the blood stream only in cases of miliary tuberculosis. On the other hand results such as these stand in need of much confirmatory evidence since in the great majority of cases no evidence of tuberculosis has been found after the inoculation of animals with the blood of tuberculous patients.

In all, abstracts are here given of 38 investigations exclusive of the present experiments. In 15 cases only microscopic results were reported, no animal tests being made. In 7 series of tests the results of animal inoculation were entirely negative. In 9 series the majority of the inoculation results were negative. In 2 reports all inoculation results are given as positive. In 3 reports a large percentage of inoculation tests was positive, while in 2 papers positive inoculation results are reported without convincing details.

In view of the mass of contradictory evidence it is important that all possible proof including cultural tests with material from the infected animal should be given in all cases of successful animal inoculation. At the same time, the experience of Liebermeister and others as to the irregular action of the same blood upon different animals must not be forgotten since one or two negative inoculation results may not be sufficient proof that no bacilli are present in a given specimen.

Judging from the negative microscopic results in the present series of specimens from so many severe cases, it must be concluded that acidfast bacilli are at least infrequent in the blood of tuberculous individuals, and while the nature of the organisms reported is a question of much interest, yet it is not a point which seems likely to prove of value in the diagnosis or prognosis of the disease.

ERRATUM.

P. 173, l. 34, "lung" should read "blood."